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DIFFERENTIAL SPUTTERING CORRECTION FOR ION MICROSCOPY USING IMAGE DEPTH PROFILING

by

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

A first-order correction for differential sputtering is made to ion micrographs using image depth profiling. This id done by first recording the ion image to be corrected, then sputtering down to the substrate while recording a series of images at the substrate's mass. A time-domain "burn-through" map showing the time each location of the sample first sputters through to the substrate is then generated. This map is used to make a linear correction for differential sputtering induced ion intensity artifacts to the initial sample images. 40 ca ion images of a radish root tip stell region on a tantalum substrate are

corrected in this fashion. Relative sputtering rates of 1.5 and 1.2 compared to the cytoplasm were found for the cell walls and nuclei, respectively.

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BRIEF: image depth profiling is used to determine relative sputtering rates of features in plant tissue analyzed by ion microscopy. Corrections for differential sputtering are made to the ion micrographs.

Ion microscopy, a form of secondary ion mass spectrometry (SIMS), is an analytical technique with the ability to generate mass resolved images of a solid sample with elemental detection limits at the parts per million level (1). A 2-20 keV primary ion beam is used to sputter the top atomic layers of the sample and secondary ions emitted from this region are extracted and mass-analyzed to produce ion images.

Qualitative interpretations of ion images have been shown to be useful in studies of elemental distributions in biological tissues (2-8). However, quantitative interpretations of ion images can be erroneous because of the problem of differential sputtering, a phenomenon first pointed out by Galle (9) and more recently by Farmer, et al (10).

The observed SIMS secondary ion intensity may be described by the equation of Morrison and Slodzian (1):

$$I = \tau C_{m} si_{po}$$
 (1)

C_m the atomic concentration of the element M (corrected for its isotopic abundance), S the total sputtering yield (number: of atoms of any kind removed per incoming particle), i_p the number of primary particles arriving per unit time and unit area on the target surface, and a_o is the analyzed area on the target surface.

Local variations in composition across a spatially heterogeneous sample's surface cause different regions of the sample to sputter at different rates. Since the observed intensity I is proportional to S, this causes erroneous ion intensity enhancements, and therefore higher apparent elemental concentrations, for the more rapidly sputtered regions. Biological tissue is a very heterogeneous material, and is thus particularly prone to differential sputtering.

An appropriate correction for differential sputtering is therefore needed for any type of quantitative work for biological tissue in particular, and spatially heterogeneous materials in general.

This paper demonstrates a first-order differential sputtering correction using digital image processing. It is performed by mounting a thin biological sample upon a substrate which produces secondary ions at a mass not observed in the sample. Images at masses of interest are recorded from the sample. The sample is then sputtered down to the substrate while a series of ion images is recorded, with increasing depth, at the substrate's mass by the image depth profiling (IDP) method of Patkin and Morrison (1). A "burn-through" map is generated from the IDP images, recording the time each location of the sample sputtered down to the substrate. This time-domain burn-through map is then used to correct the initial sample ion images for differential sputtering induced ion intensity artifacts.

EXPERIMENTAL SECTION

<u>Instrumentation:</u> Ion images were obtained with a CAMECA IMS-3f ion microscope. This ion microscope obtains images at selected mass/charge ratios which retain the original spatial relationships of the elements in the sample (12).

The microscopic image digital acquisition system (MIDAS) (13) shown in Fig. 1 was used to record images. MIDAS consists of a low-light level ISIT (intensified silicon intensified target) T.V. camera, digital frame buffer (digitally stores a 256 x 240 x 12-bit image), analog/digital converter, computer, graphics display screen, and associated computer software. The T.V. camera is focused upon the fluorescent screen

of the ion microscope. The images are acquired by the T.V. camera/frame buffer and stored by the computer. Changes from the original MIDAS description (13) include replacement of the CAMECA IMS-300 ion microscope with more sophisticated CAMECA IMS-3f and dedicated H.P. 9845T micro-computer, replacement of the DEC PDP-11/20 minicomputer and GT-40 graphics display terminal with a DEC PDP-11/34A with 256K byte RAM and two 10M byte disks, and the use of a f/2.0, 102-mm zoom lens on the T.V. camera set at 40 mm for a 50 µm field-of-view.

Computer Software: Programs were written in FORTRAN IV, RATFOR

(a structured FORTRAN IV pre-processor), and MACRO-11 assembly language.

There are four stages for differential sputtering correction by image depth profiling and digital image processing; acquisition of the image data, amendment of the data for detector and SIMS induced artifacts, image display, and the calculation of the relative differential sputtering rates of various sample features. Five separate but related programs were used for these functions.

IMAGE performs T.V. image acquisition, disk input/output of images, image display and registration.

GRFTDK acquires a series of images and transfers them to disk storage at an operator defined rate of up to one image every eight seconds.

DETCOR corrects images for spatial inhomogeneities in the image detector system. This is performed by acquiring a standard image of a smooth Si <100> single crystal, with the IMS-3f immersion lens optics slightly defocused to provide a uniformly intense ion image. The MIDAS output image is influenced by non-uniform detector response and degradation so that it appears inhomogeneous. DETCOR determines the correction factor at each pixel necessary to bring that pixel's intensity up to the maximum intensity of

the standard image, correcting for detector system heterogeneity. It then uses this factor to correct the current elemental image.

GCONV converts raw image intensities to the corresponding ion intensity using an empirically derived calibration equation.

DISCOR generates the burn-through map and then uses it to correct the image for differential sputtering. This is done by initially setting each pixel of the map to zero. The substrate images acquired by GRFTDK are then sequentially analyzed and the burn-through map updated as follows:

where $I_{x,y}^m$ is the new value of the pixel located at x,y in the burn-through map, current value is the value currently stored in that location of the burn-through map and next value is the intensity at location x,y of the image currently being examined in the GRFTDK depth profile sequence. T is the sputtering time at which the GRFTDK image being examined was acquired.

Simply, this functions as follows: Since the substrate on which the sample is mounted is being imaged, any pixel with a non-zero intensity indicates the sample has been completely sputtered away at that location.

To avoid spurious readings, however, the program waits until a location

gives a continuous series of non-zero substrate ion intensities in the IDP sequence before assigning that location a burn-through time. The burn-through time assigned is the time at which the first of these images was acquired. This procedure is repeated for every image in the IDP sequence, ultimately yielding the burn-through map which contains the time at which each pixel first sputtered through to the substrate.

At this point the burn-through map is used to perform a linear correction to the observed image intensities of the elements of interest in the sample such that:

$$I_{x,y}^{c} = I_{x,y}^{i}$$
 . burn-through time total sputtering time

where I^C is the differential sputtering corrected image, and I¹ is the detector discrimination corrected and ion intensity converted elemental image. This gives an ion image corrected, at least to a first approximation, for differential sputtering effects. This correction is based on two assumptions. The first is that the observed secondary ion intensity is proportional to the sputter rate. The second assumption requires that a given region of the sample sputters at a uniform rate throughout the course of the analysis.

Analytical Procedure: The IMS-3f instrumental conditions are shown in Table I. The parameters were chosen to optimize image quality, intensity, and sample sputtering rate. The time between images was chosen on the basis of the primary ion beam species and sample thickness.

Sample Preparation: Root tips of Raphanus sativus (radish) seedlings were selected for the study because of their well defined intracellular calcium distribution. One to 2-mm pieces of root tips were chemically fixed for 1 hr. using 5% gluteraldehyde in 0.03 M Pipes buffer [piperazine N-N-bis,(2-ethanol sulfonic acid)] at pH 6.8 (14). Root tips were washed with 0.15 Pipes buffer and then postfixed for 1 hr. in 1% osmium tetroxide in the same buffer. After dehydration in ethanol (70-90%) and propylene oxide, the tissue pieces were embedded in low viscosity resin (15). Sections were cut on distilled water using a LKB Ultrotome III and transferred onto a polished tantalum disk. Tantalum was selected as a substrate because it was not expected to be present in tissue. Sections were then stored under vacuum until analyzed in the ion microscope.

RESULTS AND DISCUSSION

Calcium in plant root tip cells was chosen for analysis due to its well defined intracellular distribution (16). A light micrograph of the stele cell region of a radish root tip section is shown in Figure 2.

Figure 3 is a ***Ca** ion micrograph clearly showing higher intensity of calcium in nuclei and cell walls than in cytoplasm. The severity of differential sputtering is indicated by Figure 4. This is not an image of ***Ca**, but of the substrate, ***I**Ta**, taken in the course of the image depth profile. These features clearly show that differential sputtering causes the nuclei and cell walls to sputter away well before the cytoplasm region. This is a serious effect which must be considered in any quantitative work.

Figure 5 is a typical burn-through map, with the lighter areas indicating the locations requiring the longest time to sputter away. The map clearly shows the cell walls as the most rapid sputtering features followed by the nuclei and then the cytoplasm. This root tip section was 0.5 µm thick and took ~5 minutes to completely burn through to the substrate.

Figure 6 presents the raw 40Ca ion image and the same image after detector discrimination, ion intensity conversion and differential sputtering corrections. Both images have been normalized to the same maximum intensity. Significant differences in the distribution of calcium in the tissue are observed and the corrected image more accurately indicates the presence of calcium in the respective features, especially in the cytoplasm. This image more closely demonstrates the physiological distribution expected in the <u>in vivo</u> situation.

Quantitatively there has been a change in the relative intensities of different features. This will obviously affect the results when converting finally from ion intensity to concentration.

The relative feature sputtering rates R_f can be calculated once the burn-through map has been generated by ratioing the average burn-through times of different features. Table II contains the average R_f values and corresponding standard deviations for cell walls and nuclei in the stele cell region of radish root tip cells under both Ar^+ and O_2^+ bombaroment. The sputter rates have been normalized to the cytoplasm sputter rate, the slowest sputtering feature.

The R_f values indicates that differential sputtering makes cell walls appear to have 1.5 times and nuclei 1.2 times more calcium (or any other element) than cytoplasm. Like results have been observed in our laboratory with corn and pea root tip cells. Galle (9) has also reported nuclei to sputter faster than cytoplasm in frog nucleated red blood cells.

The differentially sputtering nature of biological tissue may be due to its heterogeneous chemical composition. In the case of plant cells, cell wall is primarily composed of polysaccharides (cellulose, hemicell-ulose, pectine, etc.), while the nucleus is basically a proteinous material rich in DNA (deoxyribonucleic acid), RNA (ribonucleic acid), nucleoproteins, and histone proteins.

A burn-through map at a substrate mass also revealed that epoxy (where tissue is not present) was the last to sputter through, (i.e., after cytoplasm). This helps to explain the influence of epoxy in the differential sputtering of epoxy-infiltrated biological tissue. In the case of plants, cell walls are denser material than nuclei and cytoplasm, hence there may be more penetration of epoxy (mass per unit area) into the less dense cytoplasm than the denser nuclei or still more dense cell walls.

It is interesting to note that both primary beam species induce similar R_f values even though in addition to the differences in reactivity of these two species the average tissue sputter rate for an Ar^+ primary beam is greater than that observed with O_2^+ primary beam.

The $R_{\rm f}$ values indicate the possibility that our assumption of uniform sputter rate with depth may not necessarily be valid, at least when section thickness exceeds 0.5 μ m. The decrease of the $R_{\rm f}$ value with increasing sample thickness indicates significant sample homogenization. In fact, partial diffusion of 40 Ca $^{+}$ was observed to occur after several minutes of bombardment, possibly due to local sample heating or charge induced diffusion. However, the basic sample structure appears unchanged as cellular features are well defined in the 181 Ta $^{+}$ image and burn-through maps.

Factors other than differential sputtering, such as sample surface topology (17) and matrix effects (18) can also influence the observed secondary ion signal intensity, often by several orders of magnitude. Several methods of dealing with these problems have been examined elsewhere (19), and are beyond the scope of this discussion. While the influence of these factors, particularly the matrix effects, may be larger than that of differential sputtering, any comprehensive quantitation procedure will have to include corrections for differential sputtering.

In summary, differential sputtering presents a problem in the quantification of ion images, particularly for biological thin sections where cellular organelles differ widely in their chemical composition. Image depth profiling has been used to make first-order corrections for this problem. This method of correction is useful when a thin flat sample is deposited on a substrate. In addition to biological tissues this technique should also be applicable to other thin films, such as certain types of semiconductor devices and metall-urgical samples.

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CREDIT

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Table I. Instrumental Operating Conditions

Primary Ions Ar⁺, O₂⁺

Primary ion energy 10 keV

Primary ion current 3.6x10⁻¹ A/cm²

Sputtered area of sample 5.6x10⁻⁵ cm²

Secondary ion polarity positive

Transfer optics 150 µm

Vacuum at sample 3x10⁻⁸torr

Image field-of-view 50 µm

Image integration period 2 seconds

Time between images 8-40 seconds

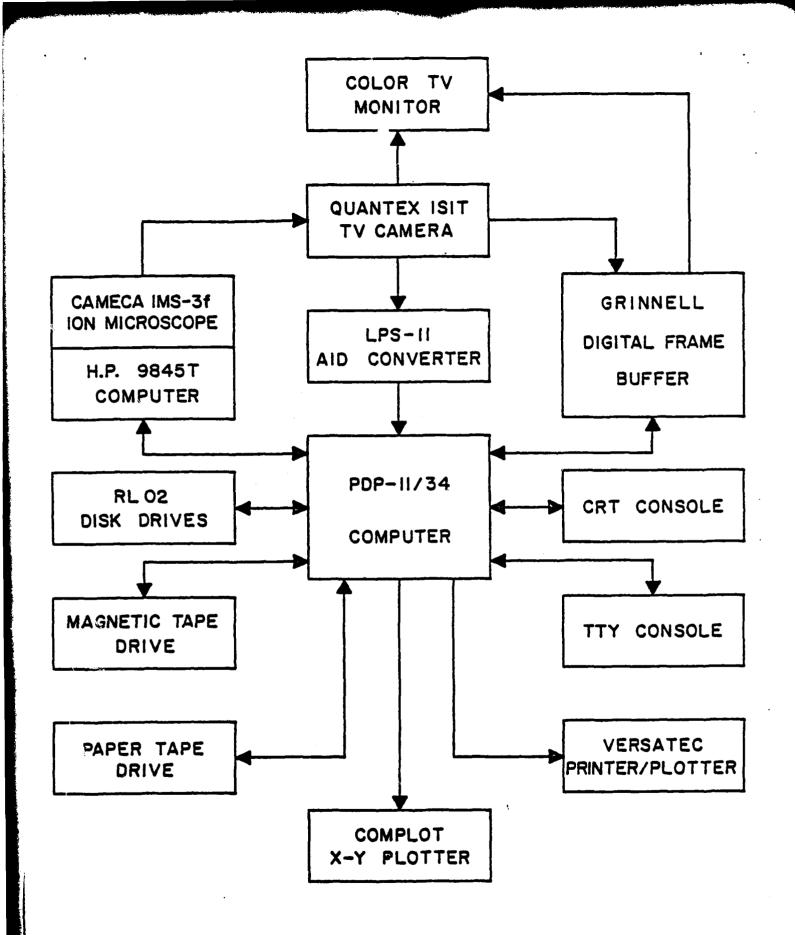
Table II. Average $R_{\mathbf{f}}$ Values by Section Thickness

R_	values
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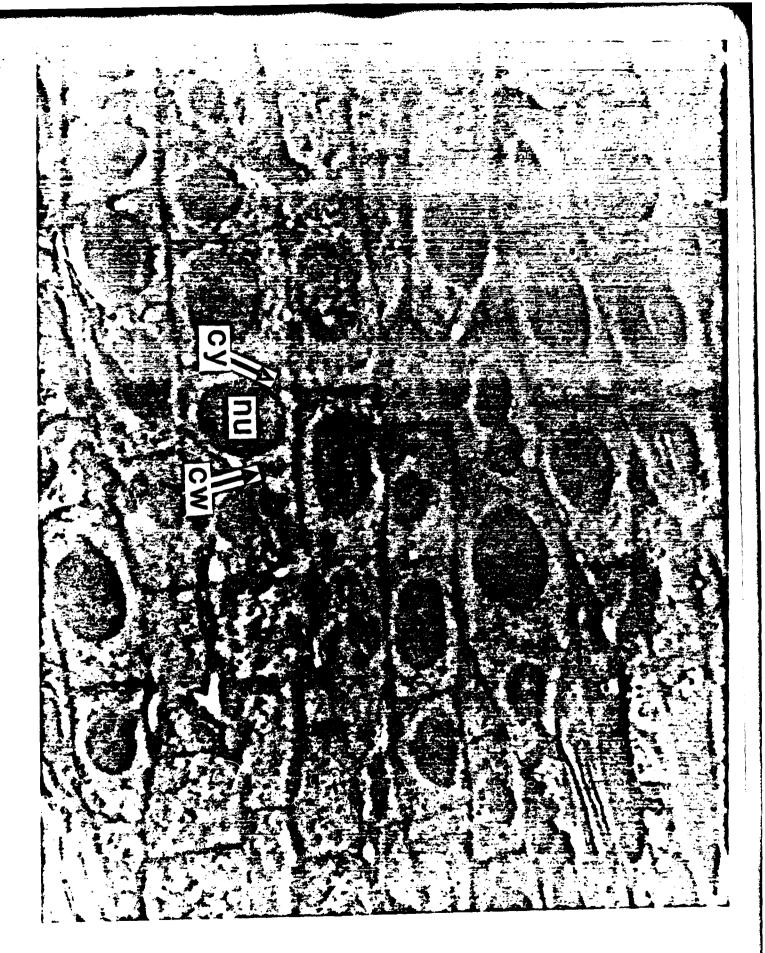
Primary Beam	Cellular Feature	0.25 µm thick section	0.50 µm thick section	1.0 µm thick section
Ar ⁺	R _f cell walls	1.51 ± 0.22	1.46 ± 0.04	1.19 ± 0.06
	R _f nuclei	1.20 ± 0.04	1.16 ± 0.02	1.09 ± 0.06
04	R _f cell walls	1.30 ± 0.08	1.50 ± 0.02	1.17 ± 0.01
	R _f nuclei	1.18 ± 0.13	1.18 ± 0.04	1.15 ± 0.01

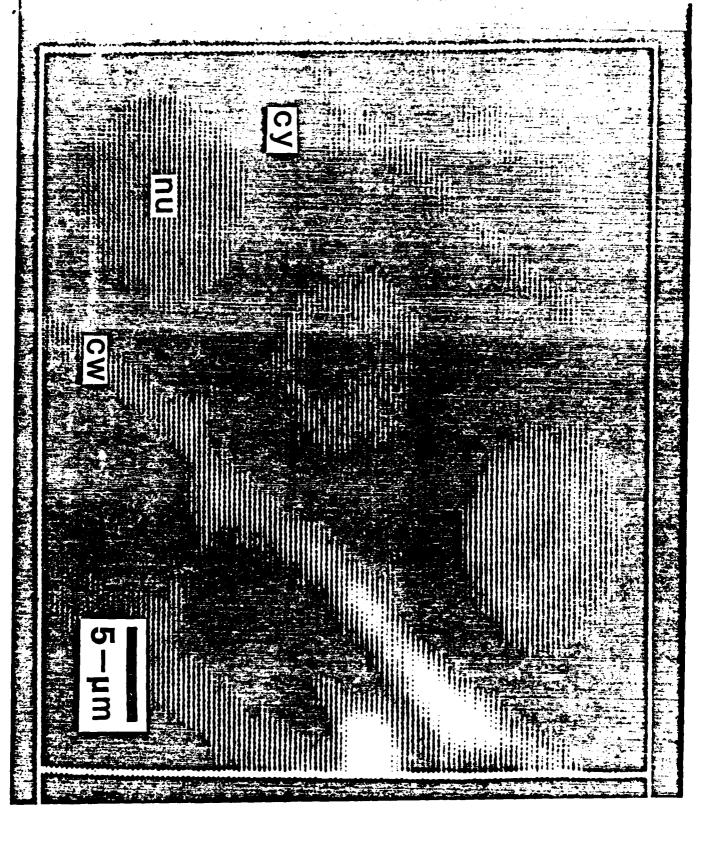
FIGURE CAPTIONS

- Figure 1. System configuration
- Figure 2. Light micrograph of radish root tip stele cell region (nu = nucleus, cw cell wall, cy = cytoplasm)
- Figure 3. *** Ca** ion micrograph of stele cells (nu = nucleus, cw = cell wall, cy = cytoplasm)
- Figure 4. 181Ta⁺ ion micrograph of substrate 150 seconds into image depth profile
- Figure 5. Time-domain burn-through map
- Figure 6. Comparison of original elemental ion image and same image after detector discrimination, ion intensity conversion, and differential sputtering corrections.

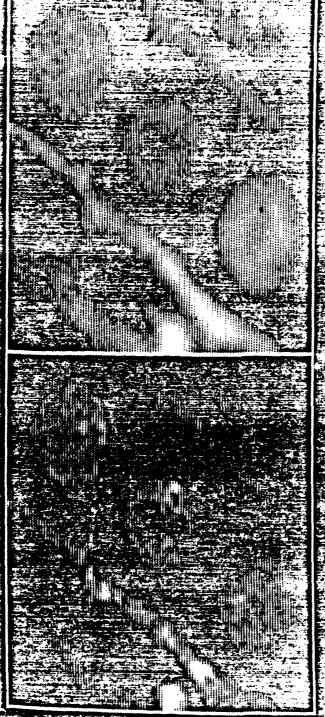


SYSTEM CONFIGURATION









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